PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION See Form PCT/IPEA/416	
P12828/KDG		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/2003/000983	12.06.2003	14.06.2002
International Patent Classification (IPC) or national classification and IPC		
C07K 14/705, C12N 5/06		
Applicant		
Cartela AB et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 4 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18.12.2003	24.09.2004
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. +46 8 667 72 88	Authorized officer Patrick Andersson/EÖ Telephone No. +46 8 782 25 00

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/2003/000983

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))

☐ publication of the international application (under Rule 12.4)

☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☐ the international application as originally filed/furnished

☒ the description:

pages 1-25 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the claims:

pages _____ as originally filed/furnished

pages* _____ as amended (together with any statement) under Article 19

pages* 1-3 (claims 1-17) received by this Authority on 26.07.2004

pages* _____ received by this Authority on _____

☒ the drawings:

pages 1-4 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☐ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/2003/000983

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-11,13,15-17,	YES
	Claims	12, 14	NO
Inventive step (IS)	Claims	1-11,13,15-17,	YES
	Claims	12, 14	NO
Industrial applicability (IA)	Claims	1-17	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

This report is based on the claims received with the letter of 2004-07-26.
The following documents are considered relevant:
D1) Camper et al. "Distribution of the collagen-binding integrin alpha10beta1 during mouse development.", 2001, Cell & Tissue Research, vol 306, pages 107-116
D2) Tiger et al, " alpha11beta1 integrin is a receptor for interstitial collagens involved in cell migration and collagen reorganization on mesenchymal non-muscle cells", 2001, vol 237, pages 116-129
D3) WO0075187
D4) WO9638482
D5) Guo Z et al., "Biological features of mesenchymal stem cells from human bone marrow", 2001, vol 114, pages 950-953
D6) Marechi K, "Isolation of human mesenchymal stem cell: bone marrow versus umbilical cord blood", 2001, vol 86, pages 1099-1100.

D1 shows alpha-10-integrin expressed together with beta1,i.e. the marker of claims 1-2. Moreover, D1 shows that alpha-10 integrin is the dominant collagen binding integrin during cartilage development and it seems to be involved in chondrogenesis.

D2 shows alpha-11-integrin expressed together with beta1, used together with alpha-10-integrin. D2 further shows that alpha-11 integrin is involved in human embryonic development and expressed in mesenchymal cells next to cartilage producing cells, indicating an involvement in cartilage repair.

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

D3 suggests the use of alpha-11 integrin as a marker for mesenchymal derived cells and stem cells, for instance to study therapeutic conditions see page 10 lines 11-23. The wording "comprising fibroblasts, muscle cells, chondrocytes, osteoblasts, mesenchymally derived cells and stem cells" is interpreted to include mesenchymal stem cells MSC since fibroblasts, muscle cells, chondrocytes and osteoblasts are derived from mesenchymal stem cells.

None of D1-D3 suggests the use of alpha-10-integrin alone or in combination as a MSC marker. Consequently, claims 1-11, 13 and 15-17 are novel, industrially applicable and considered to involve an inventive step.

D4 shows a method for isolation of mesenchymal stem cells using e.g. FACS (see D4 page 13 second paragraph). In D4 a monoclonal antibody (i.e. a compound) identifying mesenchymal stem cells is used. The MSC population created by the method of D4 is free from CD14, CD34 and CD45 presented in the present application as markers for committed lympho-haematopoetic cells or uncommitted stem cells, see table 5. Consequently claims 12 and 14 lack novelty.

Moreover, the MSC composition in D5 lack CD34 and CD45, and consequently, is detrimental to novelty of claims 12 and 14.

For the sake of completeness, present claim 12 states that a MSC composition according to said claim should be "...substantially free from molecules specific for committed lymphomhaematopoietic cells..." etc. The definition given in the description page 6, lines 35-36, seem arbitrary as it depends on how these molecules are analysed. Moreover, the results of example 3 do not indicate how non-MSC markers are analysed. Therefore even compositions such as D6 could be detrimental to novelty of claim 12, if a crude enough method of non-MSC marker analysis is applied.

CLAIMS

1. A marker for mammalian mesenchymal stem cells, comprising an integrin alpha 10 chain and/or integrin alpha 11 chain expressed on the cell surface of a mesenchymal stem cell or intracellular in a mesenchymal stem cell.

2. The marker according to claim 1, wherein the integrin alpha10 and/or integrin alpha 11 is expressed as a heterodimer in combination with an integrin beta1 chain.

3. A method for identifying a mammalian mesenchymal stem cell, the method comprising the steps of

a) providing a sample comprising a mesenchymal stem cell,

b) detecting integrin chain alpha10 and/or alpha11 expression on the cell surface of a mesenchymal stem cell or intracellular in a mesenchymal stem cell,

c) scoring the integrin chain alpha10 and/or alpha11 expression, and

d) identifying the mesenchymal stem cell according to the scoring in c) above.

4. The method according to claim 3, wherein the expression in b) above is detected by detecting the integrin alpha10 and/or integrin alpha 11 protein expression.

5. The method according to claim 3, wherein the expression in b) above is detected by detecting the integrin alpha10 and/or integrin alpha 11 mRNA expression.

6. The method according to any of claims 3-4, wherein the expression in b) above is detected by an immunoassay.

7. A method for determining whether a test compound modulates a mammalian mesenchymal stem cell differentiation, the method comprising the steps of

a) providing a mesenchymal stem cell

b) contacting the mesenchymal stem cell with a test compound, and

c) detecting a change in rate or pattern of differentiation of the mesenchymal stem cell as an indication of that the test compound modulates a mesenchymal stem cell differentiation.

8. The method according to claim 7, wherein the rate or pattern of differentiation is detected by detecting integrin chain alpha10 and/or alpha11 expression on the cell surface of said mesenchymal stem cell or intracellular in a mesenchymal stem cell according to the method in any of claims 3-6.

9. A method for producing an isolated population of mammalian cells enriched for mesenchymal stem cells relative a reference population, the method comprising the steps of.

a) providing at least a portion of a population of cells, or a portion of a reference population, comprising a mesenchymal stem cell and at least one cell other than a mesenchymal stem cells,

b) introducing into the population of cells in a) above a compound identifying the mesenchymal stem cells,

c) selecting and isolating from the population of cells in b) above the mesenchymal stem cells, thereby producing a population of cells enriched for mesenchymal stem cells.

10. The method according to claim 9, wherein the mesenchymal stem cells is identified as a mesenchymal stem cell by detecting expression of integrin alpha10 and/or alpha11 chain expression on the cell surface of said mesenchymal stem cells according to the method in any of claims 3-6.

11. The method according to any of claims 9-10, wherein the selection in c) above is performed by fluorescent cell sorting.

12. An enriched mammalian cellular population of mesenchymal stem cells, comprising at least one intact, viable mesenchymal stem cell, wherein the mesenchymal stem cell are characterised by

a) expressing an integrin alpha 10 chain and/or integrin alpha 11 chain on the cell surface of or intracellular in said mesenchymal stem cell,

b) being substantially free from expression of molecules specific for committed lymphohaematopoietic cells or uncommitted stem cells.

13. An isolated mammalian mesenchymal stem cell expressing a marker according to any of claims 1-2, obtainable by the method for producing a population of cells enriched for mesenchymal stem cells according to any of claims 9-10.

14. A mammalian cellular composition comprising the enriched cellular population according to claim 12, or the isolated mesenchymal stem cell according to claim 13.

15. Use of a marker according to any of claims 1-2, for identification of a mammalian mesenchymal stem cell.

16. Use of a marker according to any of claims 1-2, for modulating differentiation of a mammalian mesenchymal stem cell.

17. Use of a marker according to any of claims 1-2, for isolating a mammalian mesenchymal stem cell.